REMARKS

Claim Status

Claims 7, 9-18, 21, 23-27, 29-32 and 34-39 are currently pending.

Priority

In this Office Action, the Examiner reiterates from previous office action that the effective filing date for claims 14 and 29 is the filing date of application Serial No. 08/688,488, i.e., July 30, 1996, and the effective filing date for claims 35, 38 and 39 is the filing date of parent application Serial No. 08/514,875, i.e., August 14, 1995. It is noted that Applicants already accepted the above-mentioned effective filing dates in the interest of furthering the examination as mentioned in their response dated February 22, 2007.

Rejection under 35 U.S.C. § 102

Claims 7, 9-18, 21, 23-27, 29-32 and 34-39 maintain rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Fodor et al. (U.S. Pat. No. 6,610,482, "**Fodor**"). Applicants respectfully traverse this rejection.

The rejected claims are directed to a substrate with a surface comprising a microarray of distinct pre-synthesized polynucleotides, wherein the microarray has a density of 1,000 or more discrete regions of polynucleotides per cm^2 of substrate surface, and wherein polynucleotides contained in each discrete region are at least 50 subunits in length.

Fodor relates to sequencing, fingerprinting and mapping of polymers, i.e., methods of making use of microarrays (or substrates comprising microarrays). As to production of the microarrays, **Fodor** primarily describes the following two methods:

(1) The VLSIPS™ Technology (Very Large Scale Immobilized Polymer Synthesis). *See*, Col. 2, lines 58-64; Col. 12, line 17 - Col. 13, line 10; Col. 63, lines 39-44; and

(2) An automated attachment of specific probes using the caged biotin methodology (i.e., attachment of reagents in a positionally defined matrix pattern). *See*, Col. 63, lines 30-38; Col. 82, lines 28-35.

As to the microarrays so produced, Col. 3, line 2-23 of **Fodor** describes the following features:

> The present invention provides a composition comprising a plurality of positionally distinguishable sequence specific reagents attached to a solid substrate, which reagents are capable of specifically binding to a predetermined subunit sequence of a preselected multi-subunit length having at least three subunits, said reagents representing substantially all possible sequences of said preselected length. In some embodiments, the subunit sequence is a polynucleotide or a polypeptide, in others the preselected multi-subunit length is five subunits and the subunit sequence is a polynucleotide sequence. In other embodiments, the specific reagent is an oligonucleotide of at least about five nucleotides. Alternatively, the specific reagent is a monoclonal antibody. Usually the specific reagents are all attached to a single solid substrate, and the reagents comprise about 3000 different sequences. In other embodiments, the reagents represents at least about 25% of the possible subsequences of said preselected length. Usually, the reagents are localized in regions of the substrate having a density of at least 25 regions per square centimeter, and often the substrate has a surface area of less than about 4 square centimeters. (Emphasis added.)

It is clear from the above description that **Fodor** does not teach a specific length of 50 subunits or provide a microarray of polynucleotides with a length of 50 subunits for each polynucleotide. Obviously "at least three subunits", or "oligonucleotide of at least about five nucleotides, preferably at least eight nucleotides, more preferably at least about 12 nucleotides" does not equate to "at least 50 subunits" as claimed in the instant application.

In maintaining this rejection, the Examiner appears to insist December 6, 1990 be the effective filing date for **Fodor**. Applicants again contend that **Fodor** is not entitled to the benefit of that date when considering the element of "greater than 50 monomers in length" as recited in claims 40 and 56 of **Fodor** for the reasons previously presented in their responses of September 14, 2006 and February 22, 2007 and further discussed below.

The alleged effective filing date for **Fodor**, i.e., December 6, 1990 is the earliest in the priority chain as identified in the cover page of **Fodor** before there was a continuation-in-part application. In detail, **Fodor** is identified as continuation of U.S. Pat. No. 6,197,506, which is a continuation of U.S. Pat. No. 5,800,992. However, Applicants note that there appears to be an error in this priority chain, that is, **Fodor** has a specification that is substantially more than both the 6,197,506 and 5,800,992 patents in that the former patent contains 27 drawing sheets (FIGS. 1-24), whereas the latter two patents each contains only two drawing sheets (FIGS. 1-2). This error clearly suggests that **Fodor** cannot simply be a continuation of the 6,197,506 patent, which is a continuation of the 5,800,992 patent. Rather, it would appear that **Fodor** is actually a continuation-in-part of the 6,197,506 patent, which is a continuation of the 5,800,992 patent. With this finding, Applicants again contend that **Fodor** is not entitled to the benefit of December 6, 1990 as apparently alleged by the Examiner and as such, will focus the following arguments on whether U.S. Patent No. 5,800,992 ("**the '992 patent**") discloses the microarrays (or substrates comprising microarrays) of the instant application.

Same as **Fodor, the '992 patent** provides a plurality of reagents attached to a solid surface, which reagents are capable of specifically binding to a predetermined subunit sequence of a preselected multi-subunit length having at least three subunits. In some embodiments, the subunit sequence is a polynucleotide or a polypeptide, in others the preselected multi-subunit

length is five subunits and the subunit sequence is a polynucleotide sequence. In still other embodiments, the specific reagent is an oligonucleotide of at least about five nucleotides. *See*, Col. 2, line 60 thru Col. 3, line 4. **The '992 patent** does not indicate a specific length of 50 subunits or provide a microarray of polynucleotides with a length of 50 subunits for each polynucleotide. Obviously "at least three subunits", "five subunits" or "oligonucleotide of at least about five nucleotides" does not equate to "at least 50 subunits" as claimed in the instant application.

Still same as **Fodor**, **the '992 patent** also primarily describes two methods of producing microarrays, i.e., the VLSIPS™ Technology or the caged biotin methodology. Applicants present the following remarks directed to the two methods separately.

(1) *VLSIPS™ Technology*

With regard to the VLSIPS™ Technology (*in situ* synthesis), **the '992 patent** describes that it certainly allows production of ten nucleotide oligomers on a solid phase (*see,* Col. 19, lines 36-38) and that the length of oligonucleotides used will be selected on criteria determined to some extent by practical limits (*see*, Col. 20, lines 62 – Col. 21, line 7). The passage goes on as cited below:

> For example, if probes are made as oligonucleotides, there will be 65,536 possible eight nucleotide sequences. If a nine subunit oligonucleotide is selected, there are 262,144 possible permeations of sequences. If a ten-mer oligonucleotide is selected, there are 1,048,576 possible permeations of sequences. As the number gets larger, the required number of positionally defined subunits necessary to saturate the possibilities also increases. With respect to hybridization conditions, the length of the matching necessary to confer stability of the conditions selected can be compensated for.

Col. 22, lines 6-12 of **the '992 patent** further describes following limitations of the VLSIPS™ Technology in sequencing:

> As the length of the oligomer increases the number of different probes which must be synthesized also increases at a rate of a factor of 4 for every additional nucleotide. Eventually the size of the matrix and the limitations in the resolution of regions in the matrix will reach the point where an increase in number of probes becomes disadvantageous.

The above descriptions of **the '992 patent** implies that if a 50-mer oligonucleotide were selected, there would be $1{,}048{,}576^5$ possible permeation sequences. As the microarrays of **the '992 patent** comprises "a plurality of positionally distinguishable sequence specific reagents attached to a solid substrate, which reagents are capable of specifically binding to a predetermined subunit sequence of a preselected multi-subunit length having at least three subunits, said reagents representing substantially all possible sequences of said preselected length ... In other embodiments, the reagents represents at least about 25% of the possible subsequences of said preselected length" (emphasis added) (*see*, Col. 2, line 60 thru Col. 3, line 10), it is clear that even if not all possible sequences, 25% of all the possible subsequences of a 50-mer would still be an astronomic number ($1{,}048{,}576^5 \times 25\%$). Such teachings of **the '992 patent** unambiguously imply that production of an array of 50-mers (even if the array does not comprises all possible 50-mers) would be impractical as the astronomic number of probes that must be synthesized on a matrix would certainly exceed the size and resolution limits of the matrix provided by **the '992 patent**. That means, synthesis of arrays of 50-mers using the VLSIPS™ Technology would undoubtfully be disadvantageous, if not impossible.

The Examiner previously alleges, in both the Final Office Action dated Nov 22, 2006 and Advisory Action dated March 16, 2007, that arguments regarding complete n-mers are not commensurate in scope with the instant claims. Applicants respectfully disagree. Although they are not limited to complete n-mers, the instant claims encompass complete n-mers as also

acknowledged by the Examiner. In fact, as instantly claimed, the DNA sequences contained in each discrete region of the microarray can have either the same or different lengths as long as each of the DNA sequences is at least 50 subunits long. That is, the scope of the instant claims encompasses both an array of complete n-mers with n being at least 50 and an array that contain DNA sequences of different lengths (not complete n-mers) with each sequence being at least 50 subunits long. As such, arguments regarding complete n-mers are indeed relevant to the scope of the instant claims.

Also in the Advisory Action, the Examiner alleges that the arguments regarding complete n-mers are not relevant to the teaching of the prior art and that **Fodor** specifically teaches probes having 50 nucleotides. Applicants respectfully disagree. As discussed above, **the '992 patent** teaches that the microarray comprises reagents representing "substantially all possible sequences of said preselected length" or "at least about 25% of the possible subsequences of said preselected length". Such teaching indicates that the microarray of **the '992 patent** comprises reagents representing from about 25% of all possible subsequences of a preselected length to substantially all possible sequences of the preselected length. Therefore, arguments regarding complete n-mers are indeed relevant to the teachings of **the '992 patent**. As the number of all possible permeation sequences for a 50-mer is astronomic as discussed above, 25% of all possible permeation sequences would still be astronomic. That is, synthesis of arrays of 50-mers using the VLSIPS™ Technology would be undoubtfully disadvantageous as implied by **the '992 patent**. In fact, **the '992 patent** does not disclose anywhere explicitly or unambiguously that a microarray of 50-mers, whether or not comprising all possible permeation sequences, is actually produced using the VLSIPS™ Technology.

As to the Examiner's previous allegation that **Fodor** specifically teaches probes having 50 nucleotides, Applicants respectfully submit that the issue here is not whether **Fodor** teaches probes having 50 nucleotides; rather, the issue is whether the earlier applications of **Fodor** teach probes having 50 nucleotides because the Examiner apparently relies on those earlier applications for the effective filing date of **Fodor** and for the present rejection. In this regard, Applicants point out that **the '992 patent**, which is one of the earlier applications, does not disclose the subject matter of arrays of probes having 50 nucleotides (complete or not complete 50-mers) as discussed above.

In fact, the only mention of probes of greater than fifty nucleotides in the entire specification of **the '992 patent** occurs in Col. 28, lines 37-43, which describes the hybridization conditions used in fingerprinting embodiments:

> Under appropriate hybridization conditions, e.g., typically higher salt and lower temperature, the probes will hybridize irrespective of imperfect complementarity. In fact, -with probes of greater than, e.g., about fifty nucleotides, the difference in stability of different sized probes will be relatively minor.

The above passage discusses the hybridization conditions when using a microarray of probes, e.g. in the context of Southern Hybridization. A situation "with probes of greater than, e.g., about fifty nucleotides" and "different sized probes" is outlined. This outlined situation relates to the stability of different sized probes and not to an achieved microarray of 50-mer probes. As such, the above passage of **the '992 patent** does not provide a microarray of 50-mer probes as alleged by the Examiner.

In light of the above arguments regarding arrays comprising reagents of same length (complete n-mers) or different lengths (not complete n-mers), Applicants assert that their

arguments are indeed relevant to the scope of the instant claims and the teaching of the prior art. It is respectfully submitted that the above arguments are presented in two folds: (1) regarding complete n-mers (whether or not comprising all possible permeation sequences of a n-mer), that is, arrays of probes having same length of at least 50 subunits; and (2) regarding not complete n-mers, that is, arrays of probes having different lengths of at least 50 subunits. Such arguments of two folds are indeed relevant to the scope of the instant claims, which encompasses both arrays of probes having same length of at least 50 subunits and arrays of probes having different lengths of at least 50 subunits.

(2) *Caged Biotin Methodology*

With regard to the caged biotin methodology, **the '992 patent** teaches attaching specific probes onto the array surface, that is, attach pre-synthesized probes onto the array surface. *See*, Col. 27, lines 20-30. Although it teaches that isolated probe may be attached by an automated process to the matrix, **the '992 patent** does not actually teach or suggest that an array of isolated probes that are at least 50 subunits in length has actually been achieved. In fact, with the term "may be", **the '992 patent** indicates that the attachment of isolated probes to the matrix at defined positions by using the caged biotin methodology has not actually been achieved at the time of its filing. In addition, **the '992 patent** cites Serial No. 07/612,671 for the caged biotin methodology, which describes immobilization of anti-ligands such as oligonucleotides. Even if the attachment of isolated probes to the matrix using the caged biotin methodology were achieved, Serial No. 07/612,671 does not provide an achieved microarray of polynucleotides with a length of at least 50 subunits for each polynucleotide or a microarray with an achieved density of at least 1,000 discrete regions of polynucleotides per cm^2.

(3) *Conclusion*

That is, by the time of the filing of **the '992 patent**, i.e., June 25, 1996, the Fodor group was using the VLSIPS™ Technology or caged biotin methodology for providing microarrays for sequencing, fingerprinting and mapping of polymers. As discussed above, **the '992 patent** has not actually achieved a microarray of polynucleotides each having more than 50 monomeric units, wherein the polynucleotides are either synthesized *via* the VLSIPS™ Technology or pre-synthesized and individually isolated, and subsequently attached to a substrate surface using the caged biotin methodology.

As such, **Fodor**, cited by the Examiner, should not have been entitled to the priority date of **the '992 patent** at least when considering the technical feature of "greater than 50 monomers in length" as recited in claims 40 and 56 of **Fodor**. Rather, the effective filing date for claims 40 and 56 of **Fodor** should be later than the filing date of **the '992 patent**, that is, later than June 25, 1996, as further evidenced by the fact that **Fodor**'s specification includes a significant addition when compared to that of **the '992 patent** or the later 6,197,506 patent as discussed above. As such, it would appear that **Fodor** has the priority date of its own filing date of April 24, 2000.

In light of the foregoing analysis, it is obvious that one of ordinary skill in the art, at the time of the filing of **the '992 patent**, would not be encouraged to even try to produce a microarray of 50-mers using the VLSIPS™ Technology due to the increasing disadvantages and difficulties that go along with producing a microarray of increasing length of the oligomers. Also it is clear to one of ordinary skill in the art that, at the time of the filing of **the '992 patent**, no microarray of reagents of at least 50 subunits in length has actually been achieved either by using the VLSIPS™ Technology or by using the caged biotin methodology. In fact, **the '992 patent** does not disclose anywhere explicitly or unambiguously that a microarray of 50-mers (complete

n-mers) or a microarray of probes of different lengths of at least 50 subunits (not complete n-mers) is actually produced as discussed above. This is also apparent from the level of actually "achieved microarrays" in the **'992 patent** as indicated in Col. 66, line 65 thru Col. 67, line 9: what was actually achieved is the synthesis on glass of eight trimers of C and T. This is clearly not at the level of the microarray of claims 7, 21, 34 and 36 of the instant application.

Applicants further submit that, when considering the technical feature of at least 50 subunits as recited in instant claims 7, 21, 34 and 36, the instant application is entitled to at least the filing date of parent application Serial No. 08/477,809, that is, June 7, 1995. It is noted that such feature is at least supported by the parent application Serial No. 08/477,809, wherein the specification describes "(e)ach distinct biopolymer (i) is disposed at a separate, defined position in said array, (ii) has a length of at least 50 subunits" (*see*, page 7, lines 26-28), "the biopolymers are polynucleotides having lengths of at least about 50 bp" (*see*, page 26, lines 21-23), and "the polynucleotides have lengths of at least about 50 bp" (*see*, page 29, lines 5-6).

Because instant claims are at least entitled to the priority date of June 7, 1995, which date is well before the priority date for claims 40 and 56 of **Fodor** as discussed above (i.e., later than June 25, 1996), **Fodor** is not a valid prior art reference under 35 U.S.C. §102(e) for claims 7, 21, 34 and 36 of the instant application because **Fodor** has a later priority date than that of the instant application when at least considering the particular technical feature of "at least 50 subunits". Therefore, the rejection of claims 7, 9-18, 21, 23-27, 29-32 and 34-39 under 35 U.S.C. § 102(e) over **Fodor** should be withdrawn.

Claims 7, 9-15, 17-18, 21, 23-27, 29-32, 34-35 and 38-39 remain rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Winkler et al. (U.S. Pat. No. 6,677,195, "**Winkler**"). Applicants respectfully traverse this rejection.

The instant claims are directed to a substrate with a surface comprising a microarray of distinct pre-synthesized polynucleotides, wherein the microarray has a density of 1,000 or more discrete regions of polynucleotides per cm^2 of substrate surface, and wherein polynucleotides contained in each discrete region are at least 50 subunits in length.

As presented previously and reiterated herein, **Winkler** discloses a method and device for forming large arrays of polymers on a substrate. In particular, **Winkler** discloses a process of monomer-by-monomer synthesis of polymers to provide a plurality of polymer sequences on a single substrate, wherein the deposition of monomers is carried out by using a deposition device to effect the formation of a polymer in a pre-defined region. That is, the arrays of polymers are formed through an *in situ* process. Although it mentions that the process "may be" adapted to form polymers having, e.g. 50 monomers or more (emphasis added) (*see*, Col. 17, lines 53-57), **Winkler** does not actually provide a microarray with each distinct polynucleotide being at least 50 subunits in length. Such "may be" disclosure of **Winkler**'s would be a desideratum and is not an explicit and unambiguous disclosure of a microarray which would correspond to the microarray of claims 7, 21, 34 and 36 of the present application. Furthermore, the level of "achieved microarrays" in **Winkler** would appear to be not at the level of the microarray of the instant claims as **Winkler** illustrates the power of his technique through the synthesis of the complete array of six hexamer peptides from a 20 amino acid basis set (*see*, Col. 11, lines 20-48.)

Furthermore, Applicants submit that mere mention "the process may be readily adapted to form polymers having 3, 4, 5, 6, 10, 15, 20, 30, 40, 50, 75, 100 or more monomers therein" by

Winkler (*see*, Col. 17, lines 53-57), does not suggest that an array of polymers having 50 monomers was actually produced using their monomer-by-monomer synthesis process. That is, the above description does not suggest that such idea was actually reduced to practice at the time of its filing. In fact, the state of the art at the time of the filing of **Winkler** (i.e., Nov. 1992) suggests that no microarrays of polynucleotides with a length of at least 50 subunits were actually achieved as evidenced below.

Winkler and the above-discussed **'992 patent** share a common inventor (i.e., Stephen P.A. Fodor) and that **Winkler** predated the filing date of **the '992 patent** (i.e., June 25, 1996). As discussed above, **the '992 patent**, which apparently describes the later work of Fodor's group, does not actually achieve a microarray of polynucleotides of at least 50 subunits either by using the VLSIPS™ Technology or by using the caged biotin methodology. That means, at the time of the filing of **Winkler**, which is earlier than the filing date of **the '992 patent**, the state of the art indicates that no microarrays of polynucleotides of at least 50 subunits each were actually achieved.

Enabling disclosure for patent claim(s) comes from either the patent specification itself or the state of the art at the effective filing date of the patent. In this case, Since **Winkler** does not provide any enabling disclosure other than a mere mention "[i]n some embodiments the process is repeated to provide polymers with as few as two monomers, although the process may be readily adapted to form polymers having 3, 4, 5, 6, 10, 15, 20, 30, 40, 50, 75, 100 or more monomers therein" (*see*, Col. 17, lines 53-57), the state of the art is visited to determine whether a microarry of polynucleotides of at least 50 subunits, at the time of the filing of **Winkler**, was actually reduced to practice. As discussed above, the state of the art reveals that no such microarray actually existed at the time of the filing of **Winkler**.

Taking into consideration the state of the art at the time of its filing as well as the fact that it does not actually provide "an achieved microarray" with each distinct polynucleotide being at least 50 subunits in length, **Winkler** could not anticipate the present invention as claimed. Therefore, the rejection of the instant claims under 35 U.S.C §102(e) as allegedly being anticipated by **Winkler** should be withdrawn.

Claims 14, 29, 35 and 38-39 stand rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Pinkel et al. (U.S. Pat. No. 5,830,645, "**Pinkel**"). Applicants respectfully traverse this rejection.

The rejected claims are dependent from claims 7, 21, 34 or 36, which are directed to a substrate with a surface comprising a microarray of distinct pre-synthesized polynucleotides, wherein the microarray has a density of 1,000 or more discrete regions of polynucleotides per cm^2 of substrate surface, and wherein polynucleotides contained in each discrete region are at least 50 subunits in length. Although they recite further characteristics of the substrate, the rejected claims automatically carry the limitations from their base claims. That is, the rejected claims also relate to a microarray of distinct polynucleotides with a density of at least $1,000/cm^2$ and each polynucleotide being at least 50 subunits in length.

Pinkel discloses comparative fluorescence hybridization to nucleic acid arrays, which array is defined as a plurality of target elements, each comprising one or more target nucleic acid molecules immobilized on a solid surface to which probe nucleic acids are hybridized (*see* Col. 3, lines 52-54.) **Pinkel** further describes that the target nucleic acids of a target element may, for example, contain specific genes or, be from a chromosomal region suspected of being present at increased or decreased copy number in cells of interest. The target element may also contain an

mRNA, or cDNA derived from such mRNA, suspected of being transcribed at abnormal levels. Alternatively, a target element may comprise nucleic acids of unknown significance or location. *See* Col. 3, line 66 - Col. 4, line 11. In summary, **Pinkel** provides a nucleic acid array of a plurality of target elements comprising nucleic acids of known or unknown significances or originating from known or unknown chromosomal locations.

Pinkel does not teach or suggest the instantly claimed substrates. In detail, **Pinkel** discloses a nucleic acid array containing distinct target sequences from a genomic or cDNA library of same type of cells as test or control cells for comparative hybridization of probe nucleic acids to the target sequences. The nucleic acid array taught in **Pinkel** is different from the present microarray in that **Pinkel** does not teach or suggest that their nucleic acid array comprises at least 1,000 distinct target sequences per cm^2 with each sequence having at least 50 subunits in length, nor does **Pinkel** teach or suggest that the distinct target sequences contained in their nucleic acid array are individually applied to each discrete region therein. In contrast, the instantly claimed microarray comprises at least 1,000 or more distinct regions of DNA sequences per cm^2 of substrate surface, and the DNA sequences contained in each discrete region are at least 50 subunits in length. The reasoning is detailed below.

The Examiner cites Col. 4, lines 34-45 of **Pinkel** in support of her position that **Pinkel** teaches probe length of at least 50. In fact, the above-cited passage describes:

> One of skill will recognize that each target element may comprise a mixture of target nucleic acids of different lengths and sequences. Thus, for example, a target element may contain more than one copy of a cloned piece of DNA, and each copy may be broken into fragments of different lengths. <u>The length and complexity of the target sequences of the invention is not critical to the invention</u>. One of skill can adjust these factors to provide optimum hybridization and signal production for a given hybridization procedure, and to provide the required resolution among

different genes or genomic locations. Typically, the target sequences will have a complexity between about 1 kb and about 1 Mb. (Emphasis added)

That is, although describing that the complexity of the target sequences is between about 1 kb and about 1 Mb, **Pinkel** does not define probes of at least 50 subunits in length as alleged by the Examiner. Rather, the above passage teaches "the length and complexity of the target sequences of the invention is not critical to the invention", which suggests that the "length" and "complexity" of the target sequences are two different factors in the context of **Pinkel**'s invention and that the target sequences can be of any length because such factor is not critical. That is, **Pinkel** does not teach or suggest probe of at least 50 subunits in length.

In addition, the Examiner cites Col. 8, lines 61-62 in support of her position that **Pinkel** teaches a microarray having a DNA probe density of 1,000/cm^2. In fact, **Pinkel** describes on Col. 8, lines 37-67:

> To optimize a given assay format one of skill can determine sensitivity of fluorescence detection for different combinations of membrane type, fluorochrome, excitation and emission bands, spot size and the like. In addition, low fluorescence background membranes have been described (see, e.g., Chu et al., Electrophoresis 13:105-114 (1992)).
>
> The sensitivity for detection of spots of various diameters on the candidate membranes can be readily determined by, for example, spotting a dilution series of fluorescently end labeled DNA fragments. These spots are then imaged using conventional fluorescence microscopy. The sensitivity, linearity, and dynamic range achievable from the various combinations of fluorochrome and membranes can thus be determined. Serial dilutions of pairs of fluorochrome in known relative proportions can also be analyzed to determine the accuracy with which fluorescence ratio measurements reflect actual fluorochrome ratios over the dynamic range permitted by the detectors and membrane fluorescence.
>
> Arrays on substrates with much lower fluorescence than membranes, such as glass, quartz, or small beads, can achieve much better sensitivity. For example, elements of various sizes, ranging from the ~1 mm diameter down to ~1 μm can be used with these materials. Small array members

> containing small amounts of concentrated target DNA are conveniently used for high complexity comparative hybridizations since the total amount of probe available for binding to each element will be limited. Thus it is advantageous to have small array members that contain a small amount of concentrated target DNA so that the signal that is obtained is highly localized and bright. Such small array members are typically used in arrays with densities greater than $10^4/cm^2$. Relatively simple approaches capable of quantitative fluorescent imaging of 1 cm^2 areas have been described that permit acquisition of data from a large number of members in a single image (see, e.g., Wittrup et. al. Cytometry 16:206-213 (1994)).

The above passages of **Pinkel** primarily discuss sensitivity of fluorescence detection. Although mentioning that "small array members are typically used in arrays with densities greater than $10^4/cm^2$" (*see*, Col. 8, lines 61-62), **Pinkel** does not teach or suggest that their nucleic acid array comprises at least 1,000 distinct target sequences per cm^2. Neither does **Pinkel** teach or suggest that the distinct target sequences contained in their nucleic acid array are individually applied to each discrete region therein. In fact, **Pinkel** only describes that the target elements of the arrays may be arranged on a solid surface at different densities and that each target element may comprise a mixture of target nucleic acids of different lengths and sequences (*see* Col. 4, lines 30-39) with one or more copies of each sequence present in a target element (*see* Col. 2, lines 43-45). **Pinkel** is completely silent on individual disposition of each target element to each discrete region of its nucleic acid array.

In fact, Applicants believe that **Pinkel**'s teaching would not have suggested such individual and defined arrangement of each distinct gene sequence. As described in their Example 1, **Pinkel** uses avidin coated controlled pore glass "5 μm particles" as the solid support for immobilizing target sequences (*see* Col. 13, lines 21-30). Two populations of particles are used for immobilizing two different target elements each comprising multiple fragments of different lengths and sequences (*see* Col. 13, lines 10-30). The two particle populations form the

hybridization array, and a mixture of target sequences is deposited in each particle of the two populations. That is, the "smallest" disposition unit disclosed or suggested in **Pinkel** is a mixture of target nucleic acids of different lengths and sequences per particle of populations of particles comprising the target elements, not an individual DNA sequence applied to each discrete region of the microarray as claimed in the present invention. This is a teaching away from the instantly claimed microarray, which contains individually applied DNA sequences unique for each discrete region.

In view of the above remarks, **Pinkel** does not anticipate the present invention as claimed. As such, the rejection of claims 14, 29, 35 and 38-39 under 35 U.S.C. §102(e) should be withdrawn.

Rejection under 35 U.S.C. § 103

Claims 7, 9-18, 21, 23-27, 29-32 and 34-39 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Barrett et al. (U.S. Pat. No. 5,252,743, "**Barrett**") in view of **Winkler**. Applicants respectfully traverse this rejection.

The rejected claims are directed to a substrate with a surface comprising a microarray of distinct pre-synthesized polynucleotides, wherein the microarray has a density of 1,000 or more discrete regions of polynucleotides per cm^2 of substrate surface, and wherein polynucleotides contained in each discrete region are at least 50 subunits in length.

Barrett discloses immobilization of anti-ligands on surfaces. **Barrett** also discloses that the surfaces are covered with caged binding members that can be converted to activated binding members in predefined regions upon selective irradiation. In detail, **Barrett**'s substrate either comprises activated binding members at the predefined regions thereon (prior to immobilization

of anti-ligands) or comprising immobilized anti-ligands at the predefined regions thereon (post immobilization of anti-ligands). Although mentioning oligonucleotides as one of the other examples of anti-ligands, **Barrett** does not teach or suggest an array of DNA sequences with a density of at least 1,000/cm^2 and each sequence being at least 50 subunits in length.

The Examiner alleges that **Barrett**'s array surface has a density of about 400 (10,000) regions/cm^2 by citing the passage in Col. 20, lines 20-25, Example 1 and Fig. 4. Applicants respectfully disagree.

First, the Examiner's above citation of Col. 20, lines 20-25 of **Barrett** discusses biotinylated anti-ligands having specific binding affinity for avidin and has nothing to do with array density. Rather, **Barrett** describes the sizes of exposed area on the substrate as well as spaces between activated regions in Col. 18, line 67 thru Col. 19, line 4:

> In some embodiments, the exposed area is less than about 1 cm^2 or less than about 1 mm^2. In preferred embodiments the exposed area is less than about 10,000 μm^2 or, more preferably, less than about 100 μm^2. Spaces between activated regions are not critical and will generally be greater than about 1 μm.

The above description only provides the size of each of the irradiated regions on the substrate surface. It appears that the Examiner has interpreted the size of the illumination checkerboard-pattern mask (i.e., 500 μm × 500 μm) as the overall size of the glass microscope slide. This interpretation is obviously erroneous as illumination mask could be bigger than the slide and as such, the size of the mask is not necessarily the same as that of the slide. This notion is also supported from the fact that a glass microscope slide is normally rectangular not square, and that the above-specified size of 500 μm × 500 μm clearly indicates that the illumination mask is in the shape of a square. As such, **Barrett** is silent on the overall size of the substrate surface. Without knowing the size of the surface, one cannot determine what the density might

be for **Barrett**'s substrate surface. Therefore, **Barrett** does not teach the density element as claimed in the instant application.

Secondly, the Examiner's above citation of "Example 1" appears to be an error as no such example exists in **Barrett**. Applicants assume that the Examiner meant "Example J" as this example describes that the glass microscope slide prepared with caged-biotin "was mounted on a custom flow cell and illuminated through a 500 μm × 500 μm checkerboard-pattern mask" (*see*, Col. 29, lines 6-10). Even so, Applicants respectfully submit that **Barrett** does not disclose any information about the density, let alone a density of about 400 (10,000) regions/cm^2 as alleged by the Examiner.

In addition, the Examiner alleges that **Barrett** "clearly suggests oligonucleotides of at least 50 subunits" because it teaches that "the polynucleotides are greater than 1kD" (Column 20, lines 45-47, 57) as well as suggests that "other various large molecules ... are 'typically greater than about 1kD'" (Column 20, lines 45-47, 57). In response, Applicants respectfully disagree.

Barrett describes in Col. 20, lines 41-60:

> Anti-ligands that mediate a biological function on binding with particular ligand(s) are of most interest. Suitable anti-ligands include relatively small, single molecules, such as cofactors, which show specific binding properties. Typically, anti-ligands will be greater than about 100 daltons in size and more typically will be greater than about 1kD in size. Other examples of anti-ligands include, but are not restricted to, the common class of receptors associated with the surface membrane of cells and include, for instance, the immunologically important receptors of B-cells, T-cells, macrophages and the like. Other examples of anti-ligands that can be investigated by this invention include but are not restricted to hormone receptors, hormones, drugs, cellular receptors, membrane transport proteins, steroids, peptides, enzymes, substrates, cofactors, vitamins, lectins, sugars, oligonucleotides, oligosaccharides, viral epitopes, antigenic determinants, glycoproteins, and immunoglobulins, e.g., monoclonal and polyclonal antibodies. (Emphasis added)

The above passage of **Barrett** teaches that suitable anti-ligands are relatively small, although typically, anti-ligands will be greater than about 100 daltons in size and more typically will be greater than about 1kD in size. Such description does not suggest "oligonucleotides of at least 50 subunits" as alleged by the Examiner because (1) size of anti-ligands (particularly in reference to proteins) being greater than 100 daltons does not translate into size of oligonucleotides being at least 50 subunits; and (2) the above description of **Barrett** does not state that the anti-ligands suitable for its substrate are actually greater than about 100 daltons in size, or more typically, greater than about 1kD in size. Rather, **Barrett** teaches that relatively small anti-ligands are suitable and that anti-ligands, typically, are greater than about 100 daltons in size, or more typically, greater than about 1kD in size.

Furthermore, **Barrett** does not disclose any specific species of oligonucleotides as the suitable anti-ligands, which notion is also acknowledged by the Examiner. Even if various species were obvious in view of a teaching of an oligonucleotide genus as alleged by the Examiner, **Barrett** does not teach or suggest the instantly claimed substrates because it does not teach an array of distinct polynucleotides with a density of at least 1,000/cm^2 and each polynucleotide being at least 50 subunits in length.

The Examiner further cites **Winkler** in support of her allegation that nucleic acid probes of at least 50 subunits were well known and preferred in the art at the time the claimed invention was made. In response, Applicants respectfully disagree and submit that **Winkler** does not provide a microarray of distinct polynucleotides with each polynucleotide being at least 50 subunits in length as discussed above.

In view of the above remarks, even if motivated to combine the teachings of **Barrett** and **Winkler**, one skilled in the art would still not have made the present invention as claimed. As

such, the rejection of claims 7, 9-18, 21, 23-27, 29-32 and 34-39 under 35 U.S.C. §103(a) should be withdrawn.

Double Patenting

Claims 7, 9-18, 21, 23-27, 29-32 and 34-39 are provisionally rejected on the ground of non-statutory obviousness-type double patenting as being unpatentable over claims 36-47 of co-pending application Serial No. 08/688,488. In response, Applicants wish to delay submission of an appropriate terminal disclaimer until allowance of either the present application or co-pending application Serial No. 08/688,488 is warranted.

*****

This paper is filed timely. No fees are believed to be due. However, should any fees be required for any reason relating to this document, the Commissioner is authorized to deduct such fees from Howrey LLP Deposit Account No. 08-3038/12665.0009.CNUS01.

Respectfully submitted,

J. Wendy Davis, Ph.D.
Reg. No. 46,393

Agent for Assignee
THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY

Customer No. 23369

HOWREY LLP
1111 Louisiana, 25th floor
Houston, TX 77002
(713) 787-1512 (Direct)

Date: October 23, 2007

EXPRESS MAIL MAILING LABEL

NUMBER: **EV 958323605 US**
DATE OF DEPOSIT: October 23, 2007

I hereby certify that this paper or fee is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria VA, 22313-1450.

Paula S. Linkhart